PROSPECT
Journal of Cellular Biochemistry 106:975–983 (2009)

Journal of Cellular Biochemistry

Statins, Stem Cells, and Cancer

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ABSTRACT

The statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) were proven to be effective antilipid agents against cardiovascular disease. Recent reports demonstrate an anticancer effect induced by the statins through inhibition of cell proliferation, induction of apoptosis, or inhibition of angiogenesis. These effects are due to suppression of the mevalonate pathway leading to depletion of various downstream products that play an essential role in cell cycle progression, cell signaling, and membrane integrity. Recent evidence suggests a shared genomic fingerprint between embryonic stem cells, cancer cells, and cancer stem cells. Activation targets of *NANOG*, *OCT4*, *SOX2*, and *c-MYC* are more frequently overexpressed in certain tumors. In the absence of bona fide cancer stem cell lines, human embryonic stem cells, which have similar properties to cancer and cancer stem cells, have been an excellent model throwing light on the anticancer affects of various putative anticancer agents. It was shown that key cellular functions in karyotypically abnormal colorectal and ovarian cancer cells and human embryonic stem cells are inhibited by the statins and this is mediated via a suppression of this stemness pathway. The strategy for treatment of cancers may thus be the targeting of a putative cancer stem cell within the tumor with specific agents such as the statins with or without chemotherapy. The statins may thus play a dual prophylactic role as a lipid-lowering drug for the prevention of heart disease and as an anticancer agent to prevent certain cancers. This review examines the relationship between the statins, stem cells, and certain cancers. J. Cell. Biochem. 106: 975–983, 2009.

KEY WORDS: STATINS; APOPTOSIS; CELL PROLIFERATION; ANGIOGENESIS; HUMAN EMBRYONIC STEM CELLS

Statins, the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors are a class of drugs that inhibit the rate-limiting step in the cholesterol biosynthetic pathway. Cholesterol is an important structural component of cell membranes and our day-to-day physiological requirements are met either by endogenous synthesis or exogenous supply. Increases in lipid levels lead to atherosclerosis and narrowing of the blood vessels, which in turn may affect the coronary blood supply to the heart, brain, and peripheral circulation leading to morbidity or mortality. The Framingham study which started in 1948 as a long-term epidemiological analysis of coronary heart disease (CHD) clearly identified the link between raised blood cholesterol and increased CHD risk which remains a major cause of death in industrialized nations [Murray and Lopez, 1997]. Statins, by inhibiting cholesterol biosynthesis, emerged as a principal agent in lowering the incidence of cardiovascular disease. However, any compound leading to depletion of cholesterol, which is the main structural component of cell membranes, in turn affects various cellular events and impairs homeostasis. This was further supported by observed consistent increases in non-cardiovascular-related mortality following statin therapy and the association of low cholesterol levels with increase in cancer risks [Oliver, 1991]. This fact led to screening of the effect of statins on the incidence of various cancers as part of the controlled trial studies on statins. Various studies have been reported describing the association of statins with either an increase or decrease in incidence of various cancers [Kwak et al., 2000; Wong et al., 2002]. These differences in association of statins with cancer might be due to the influence of other predisposing factors such as diet, smoking, and drugs as well as the specific type and site of cancer, and the duration of the study period given the latency period of the occurrence of different tumors.

The role of statins extends beyond its lipid-lowering effects, as they are known to improve endothelial functions, participate in plaque stabilization, immunomodulation, antioxidant activity, and also act as anti-inflammatory and anticancer agents. These properties together with a high safety profile have made statins more attractive and continue to be a widely prescribed drug. In the United Kingdom, simvastatin has acquired ''over-the-counter'' status from its earlier status of purchase by prescription only.

The recent excitement in human embryonic stem cell (hESC) biology as a panacea for the treatment of many incurable diseases in regenerative medicine has stimulated tremendous interest in cancer

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Received 12 January 2009; Accepted 14 January 2009 • DOI 10.1002/jcb.22092 • 2009 Wiley-Liss, Inc.
Published online 17 February 2009 in Wiley InterScience (www.interscience.wiley.com).

stem cell (CSC) biology. More interestingly, hESCs behave like neoplastic cells and given their pluripotent nature they produce teratomas thus making them an ideal model to study the role of stem cells and anticancer agents in cancer biology. Until such time as CSC lines are freely available for cancer studies, studies on hESCs will shed tremendous insight as to the pathogenesis and treatment of certain cancers. In fact, the recent claims of the existence of CSCs within tumor tissues as being responsible for the initiation, progression, and recurrence of tumors has opened up new avenues in cancer research [O'Brien et al., 2007]. Several common properties exist between CSCs and hESCs, namely their self-renewal potential, high proliferative capacity, long population doubling time, increased telomerase activity, high nuclear to cytoplasmic ratios, and induction of tumors in immunodeficient mice. Additionally, both stem and cancer cells share same signaling mechanisms [Taipale and Beachy, 2001]. It thus may be useful to study new compounds that will specifically target and destroy CSCs which once eliminated may prove beneficial against tumor progression and recurrence preferably with minimal or no side effects.

With statins gaining more attention for their effects against cancer cells, the present review aims to bring together the reports form various prospective and retrospective case–control studies, in vitro and in vivo studies, and other additional insights into the relationship between stemness, statins, and cancer cells.

STATINS—A BRIEF HISTORY OF THE HMG-CoA REDUCTASE INHIBITORS

Initially, several lipid-lowering drugs such as nicotinic acid, cholestyramine, estrogens, and dextrothyroxine were in clinical use but due to their associated side effects none were considered ideal. The demonstration that the feedback suppression of hepatic cholesterol synthesis by dietary cholesterol is mediated by HMG-CoA reductase in the conversion of HMG-CoA to mevalonate, paved way for the later development of HMG-CoA reductase inhibitors. Mevastatin was the first active compound identified from *Penicillium citrinum* to inhibit HMG-CoA reductase, and its inactive lactone form can be converted to a water-soluble acid form naturally in the liver or by treatment with an alkali to become an active compound. Mevastatin was found to be ineffective in mice and in rats even at high doses for periods as long as 5 weeks with no reduction in plasma cholesterol. However, its effectiveness came to light when tested in a rat model with increased HMG-CoA reductase activity followed by experiments in laying hens and later followed by clinical trials in patients. Success with mevastatin encouraged continued search for similar compounds that led to the isolation of lovastatin [Alberts et al., 1980]. Modification of these natural statins led to the development of more potent semi-synthetic and synthetic statins (Table I). Simvastatin and pravastatin belong to the semi-natural statins while fluvastatin, atorvastatin, cerivastatin, crilvastatin, and pitavastatin belong to the synthetic statins.

THE CHOLESTEROL BIOSYNTHETIC PATHWAY

Understanding cholesterol biosynthesis from its precursor mevalonate will provide insights for the observed multiple effects of these HMG-CoA reductase inhibitors (Fig. 1). Statins reduce serum cholesterol levels by competitively inhibiting HMG-CoA reductase, the rate-limiting enzyme in the mevalonate pathway. Mevalonate, apart from being involved in cholesterol synthesis, is also involved in the synthesis of isoprenyl proteins, dolichol, and ubiquinone that play several key cellular functions such as cell signaling, proliferation, growth, and respiration. Statins also exert effects that are independent of the mevalonate pathway viz., immunomodulatory effects by inhibition of leukocyte function antigen-1 [Weitz-Schmidt et al., 2001] and free oxygen radical scavenging by metabolites of statins [Aviram et al., 1998]. The isoprenoid molecules, farnesyl pyrophosphate (FPP), and geranylgeranylpyrophosphate (GGPP) are necessary for post-translational modification or isoprenylation of a variety of proteins such as Ras and Rho that are involved in cell motility through cytoskeletal reorganization [Jackson et al., 1997]. Dolichyl phosphate, an oligosaccharide donor in the glycoprotein synthesis is involved in the regulation of cell growth through N-linked glycosylation of the insulin-like growth factor-1 (IGF-1) receptor. Ubiquinone is associated with mitochondrial respiration and its depletion has been suggested to cause myopathy, the most serious side effect observed with some synthetic statins although not supported with definitive evidence [Nakahara et al., 1998].

STATINS AND INCIDENCE OF CANCER

Numerous studies on statins and various cancers have been carried out by different research groups. The best information on the

TABLE I. History and Development of Some Statins

Name of statin	Type	Isolation/modification	Research group/company
Mevastatin (lipophilic)	Natural	*Penicillium citrinum*	Endo et al. [1976]
		Penicillium brevicompactum	Brown et al. [1976]
Lovastatin (lipophilic)	Natural	*Aspergillus terreus*	Alberts et al. [1980]
		Monacus rubber	Endo [1979]
Simvastatin (lipophilic)	Semi-synthetic	A lovastatin analog with an additional methyl group	Hoffman et al. [1986]
Pravastatin (hydrophilic)	Semi-synthetic	A mevastatin analog with an additional hydroxyl group by microbial transformation (*Streptomyces carbophilus*)	Tsujita et al. [1986]
Fluvastatin (lipophilic)	Synthetic	Indolyl derivative	Kathawala [1991]
Atorvastatin (lipophilic)	Synthetic	Substituted H-pyrrole compound	Sliskovic et al. [1991]
Cerivastatin (lipophilic)	Synthetic	Pyridine derivative	Angerbauer et al. [1994] (withdrawn from clinical use in 2001)
Crilvastatin (lipophilic)	Synthetic	Pyrrolidone derivative	Pan Medica
Rosuvastatin (hydrophilic)	Synthetic	Pyridine derivative	AstraZeneca
Pitavastatin (lipophilic)	Synthetic		Nissan-Kowa

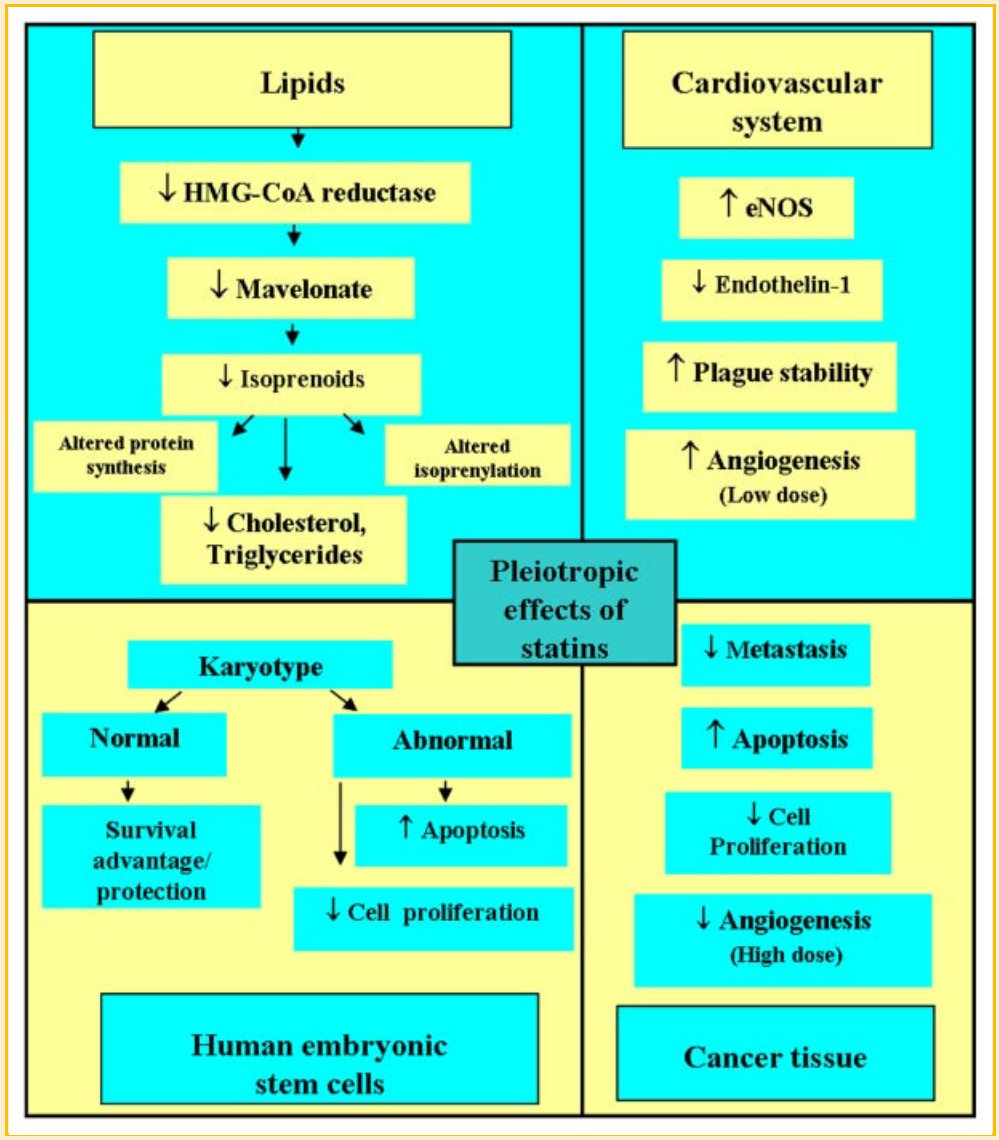

Fig. 1. Mechanism of cholesterol inhibition by statins and their effects on the cardiovascular system, cancer prevention, and human embryonic stem cells. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

association of statins with cancer is obtained by pooling of data from observational, in vitro and in vivo studies and meta-analyses of several large trials involving patients with cardiovascular disease.

A probable link of statins with cancer emerged following randomized controlled trials to study the role of statins in cardiovascular disease prevention. Evaluation of approximately 29,000 patients from 16 randomized placebo-controlled studies followed over a period of 3.3 years showed no association of statin use and risk of cancer [Hebert et al., 1997]. Bjerre and LeLorier [2001] in another meta-analysis from five large trials in which patients were followed for 5 years also found no association between statin use and risk of cancer. Although these studies included large sample sizes, the duration of study was not long enough to identify an association as some of the cancers have long latency periods before manifestation.

Since statins bring about substantial reduction in cholesterol level and other downstream mevalonate products, it appears plausible that perturbations in the cell structural and functional components might predispose to cancer development.

In contrast to the above studies, in a study that involved 542 cases of incident cancers and 5,420 controls from the Quebec Administrative Health Database followed-up to 2.7 years, there was a significant 28% reduction in the risk of cancer with statins [Blais et al., 2000]. Graaf et al. [2004] reported a significant 20% reduction in cancer risk following statins in a Dutch database study involving 3,129 cases and 16,976 controls. Sata et al. [2004] demonstrated that statins led to augmentation of the collateral vessels following ischemia but did not promote cancer and atherosclerosis. There is growing evidence that statins when used alone or in combination with other antitumor therapy are associated with decrease in the risk

of cancer [Jackobisiak and Golab, 2003]. Thus, it could be seen that statins are gaining popularity as anticancer agents against various cancers including colorectal cancer, skin cancer (melanoma), prostate cancer, and breast cancer. Current research is revealing important new statin targets (LFA1, RHO isoforms, and post-prenylation enzymes) leading to the development of more-specifically targeted agents for cancer prevention [Demierre et al., 2005].

STATINS AND SPECIFIC CANCER TYPES

COLORECTAL CANCER

Colorectal cancer is a major health concern and although mostly preventable with dietary habits and early diagnosis, the mortality rate still remains high. Following early findings that simvastatin had consistent chemopreventive effects against colon carcinogenesis, many studies have explored the effect of statins and found varied results. In a case–controlled study over 5 years in a population that used pravastatin and simvastatin, Poynter et al. [2005] reported a reduction in the relative risk of colorectal cancer by 47%. In a recent meta-analysis of 18 studies involving more than 1.5 million patients [Bonovas et al., 2007], the authors concluded that although statins do not strongly reduce the risk of colorectal cancer, a moderate reduction in risk could not be ruled out with higher doses of statins. Considering the latency period associated with colorectal cancers, which vary from 10 to 20 years before detection, intervention, and clinical presentation, these studies may appear inconclusive with respect to statin dose and duration. Placebo-controlled randomized clinical trials need to be undertaken to address these issues to identify the real effects of statins in colorectal cancer.

Studies on animal models of colorectal cancer and in vitro studies demonstrated the beneficial effects of statins. The chemo-preventive efficacy of farnesol and lanosterol (the feedback inhibitors of HMG-CoA reductase) on azoxymethane-induced colonic aberrant crypt foci in rats indicated that statins might by useful in the prevention of colorectal cancer [Rao et al., 1999]. Additionally, atorvastatin when administered together with low doses of celecoxib inhibited intestinal tumorigenesis in $APC^{(min)}$ mice which is a genetically predisposed mouse model of colorectal cancer [Swamy et al., 2006].

PROSTATE CANCER

A case–controlled study by Shannon et al. [2005] involving 100 prostate cancer cases and 202 clinical controls found statins to be associated with 50% reduction in the risk of prostate cancer (odds ratio = 0.24, 95% CI: 0.11–0.53), and this was directly proportional to the dose and duration of statin use. Clinical improvement and biochemical progression-free survival after brachytherapy for clinically localized prostate cancer was reported with atorvastatin [Moyad et al., 2005]. Lovastatin was not effective in the in vivo control of prostate cancer in a transgenic male mouse model although it did inhibit the growth of prostate cancer cells in vitro [Shibata et al., 2003]. Lovastatin induced senescence and G1 cell cycle arrest in human prostate cancer cells in vitro that was reversed by addition of GGPP or mevalonate, but not FPP [Lee et al., 2006].

BREAST CANCER

A recent study that included data over 6.4 years, 2,707 breast cancer cases were seen among 92,788 women of which 7.4% had used statins for at least 1 year. This study also did not support an association between statin use and breast cancer risk [Boudreau et al., 2004]. In contrast, a prospective cohort study of early-stage breast cancer survivors with post-diagnosis statin use showed a decreased risk of breast cancer recurrence (RR = 0.66, 95% CI: 0.39–1.13). Most of the statins used were lipophilic and the mean duration of statin use in the study cohort was 1.96 years [Kwan et al., 2008].

In vivo results in a mouse ErbB2(+) breast cancer model showed that lipophilic statins (fluvastatin, lovastatin, and simvastatin) significantly inhibited the growth of a mouse mammary carcinoma but pravastatin (hydrophilic) had no inhibition of tumor growth [Campbell et al., 2006]. In vitro lovastatin was demonstrated to increase gap junctional intercellular communication, influence intracellular calcium levels, and alter cell signaling leading to inhibition in MCF-7 (breast adenocarcinoma) cells [Wei et al., 2007]. Inducible nitric oxide-mediated pro-apoptotic, tumoricidal, and antiproliferative effects of both fluvastatin and simvastatin in MCF-7 cells were also reported [Kotamraju et al., 2007].

STATINS AND THEIR PROBABLE ANTICANCER MECHANISMS

Recently, studies show statins to be beneficial as anticancer agents. Their antitumor effects may be due to inhibition of cell proliferation, promotion of apoptosis, inhibition of angiogenesis, prevention of metastasis, improvement of immunity, or possibly targeting the CSC population (Fig. 1).

INHIBITION OF CELL PROLIFERATION

Various statins inhibit cancer cell proliferation in vitro and in vivo through HMG-CoA reductase inhibition and depletion of isoprenoids. Cell proliferation inhibition varies depending on the sensitivity of the different statins and cell types with which they interact. In general, cancer cells in a variety of organs appear to be sensitive to statins [Shibata et al., 2003; Horiguchi et al., 2004; Lee et al., 2006]. Inhibition of cell proliferation due to HMG-CoA reductase inhibitors has been suggested to be due to prevention of transition from G1 to S phase in the cell cycle, down-regulation of cyclin-dependent kinases that facilitate cell cycle progression or up-regulation of cell cycle inhibitors [Lee et al., 2006]. This inhibition of cell proliferation could be reversed by the availability of free mevalonate, FPP, or GGPP, indicating that the observed effects of statins are indeed mediated by HMG-CoA reductase inhibition.

PROMOTION OF APOPTOSIS

Statins have been shown to induce apoptosis through extrinsic or intrinsic pathways leading to cell death. Up-regulation of pro-apoptotic proteins such as BAX combined with down-regulation of the antiapoptotic protein BCl2 has been demonstrated to contribute to cancer prevention [Agarwal et al., 1999]. Shibata et al. [2003] reported a p53-independent mitochondrial-mediated apoptosis following lovastatin exposure in a mouse mammary carcinoma.

The sensitivity of different cell types to stains via apoptosis varies, with acute myeloid leukemic and neuroblastoma cells being more sensitive than acute lymphoblastic leukemic cells [Dimitroulakos et al., 1999]. Apoptotic events following statin therapy become evident following treatment at higher concentrations unlike cell inhibition that could be observed at lower concentrations.

INHIBITION OF ANGIOGENESIS AND METASTASIS

Vascular supply is an essential component of cancer cell survival, progression, and metastasis. The statins are reported to increase bone marrow-derived endothelial progenitor cells, which are involved in the neovascularization process of ischemic tissues [Murohara et al., 2001]. Low-dose statins enhance angiogenesis via activation of endothelial nitric oxide synthase [Kureishi et al., 2000]. Since these studies show that statins help to increase blood supply it may be considered beneficial in ischemic disease of the heart but detrimental in cancer as increases in vascularity will support cancer growth and progression. However, Weiss et al. [2002] categorically demonstrated that high doses of statins reduced tumor growth associated with tumor vascular inhibition, which was lipid independent and reversible with mevalonate and GGPP.

It has been claimed that the Rho family of small GTPases play an essential role in cancer metastasis, and Collisson et al. [2003] demonstrated that overexpression of Rho proteins increased the invasive potential of melanoma cells and atorvastatin caused disruption of stress fibers as a result of Rho inhibition hence decreasing the metastatic potential in melanoma cells. Similarly, as a result of Rho inhibition, statins were shown to reduce the invasive potential of breast carcinoma cells [Farina et al., 2002].

In general, there seems to be considerable overlap of different mechanisms by which statins exert their beneficial effects. In addition to the above-mentioned mechanisms, the anti-inflammatory, immunomodulatory, and radiosensitizing properties of statins may further contribute to the inhibition of cancer.

STATINS AND STEM CELLS

Stem cells are cells that are capable of self-renewal and can differentiate into specialized cell types. They can be classified as embryonic, fetal, umbilical, or adult stem cells. The presence of a CSC within a tumor that can re-grow the tumor and hence is the cell that must be destroyed if the entire tumor and metastases are to be controlled by treatment has been an interesting hypothesis put forward recently by many workers. This hypothesis has been supported by the fact that serial transplantation of animal tumors often leads to a reduction in the number of cells required to transplant the tumor as the number of transplant generations increases, suggesting selection of CSCs [Hill, 2006]. However, the case that a small proportion of cells in solid tumors are specific CSCs and that these cells can be successfully identified and isolated has not been proven as yet [Hill, 2006].

CSCs have been defined as a small group of cancer cells within a cancer that constitute a reservoir of self-sustaining cells which have the exclusive ability of self-renewal and cause the heterogeneous lineages of cancer cells that comprise the tumor [Clarke and Fuller, 2006]. It has been claimed that such cells should be really called "cancer-initiating cells" until such time that such cells are fully characterized and shown to possess all the features of "stemness" like other stem cells [Hill and Perris, 2007]. Identifying their presence, properties, and actual function to generate the other cancer cells within a tumor will help to target treatment at the CSC itself with more promising therapeutic outcome. Several surface CD markers (CD24, CD44, CD133, CD166) and dye efflux assays have been used to identify putative CSCs in cancer cell lines in vitro and fresh cancer biopsies [O'Brien et al., 2007; Ricci-Vitiani et al., 2007]. However, such markers and assays are not all that reliable in confirming the existence of a bona fide CSC to be the gold standard for identification. There have been uncertainties as to whether such putative CSCs are therapeutically sensitive to certain agents and whether they are a fixed or moving target than can evade destruction by the agent. The direct action of the statins on such CSCs has not been attempted as yet because of a lack of specific markers to identify them as bona fide stem cells within a cancerous cell population.

It has also been proposed that the differences between CSCs and normal stem cells are their degree of dependence on the stem cell niche, which is a specialized environment in which stem cells reside [Li and Neaves, 2006]. The stem cell niche is important in maintaining "stemness" and preventing tumorigenesis by providing inhibitory signals for proliferation and differentiation, and transient signals for stem cell division to support ongoing tissue regeneration. This balance is important for maintenance of stem cell function. These workers suggested that CSCs may arise from an intrinsic mutation leading to self-sufficient cell proliferation and/or may involve deregulation or alteration of the niche by dominant proliferation-promoting signals.

Recently, Jaksch et al. [2008] showed cell cycle-dependent variation of a CD133 epitope in hESC, colon cancer, and melanoma cell lines. Reactivity with an antibody (AC133) to a glycosylated form of CD133 (a pentaspan transmembrane glycoprotein expressed in several stem cell populations and cancers) has been used for cell enrichment with tumor-initiating activity in xenotransplantation assays. Jaksch et al. [2008] showed by fluorescent-activated cell sorting (FACS) that increased AC133 reactivity in hESCs, colon cancer, and melanoma cells was correlated with increased DNA content and that the least reactive cells were in the G1–G0 portion of the cell cycle. The association of AC133 with actively cycling cells was suggested to contribute to the basis for enrichment for tumor-initiating activity. CSCs may be caused by disturbance of self-renewal and differentiation occurring in multipotential stem cells, tissue-specific stem cells, progenitor cells, mature cells, and cancer cells [Wu, 2007].

CANCER STEM CELLS AND EMBRYONIC STEM CELLS

Through the property of self-renewal, Reya et al. [2001] postulated that striking parallels could be found between stem cells and cancer cells. These authors suggested that tumors may often originate from the transformation of normal stem cells, similar signaling pathways may regulate self-renewal in stem cells and cancer cells, and cancer cells may include "CSCs"-rare cells with indefinite potential for self-renewal that drive tumorigenesis.

Recent studies have shown that while CSCs and ESCs undergo indefinite self-renewal they also share common pathways of "stemness." Two independent groups demonstrated that ESCs and multiple types of human cancer cells shared gene expression patterns that were repressed in normal differentiated somatic cells [Ben-Porath et al., 2008; Wong et al., 2008]. They identified two predominant gene modules that distinguish ESCs from adult tissue stem cells. The ESC-like transcriptional program was activated in diverse human epithelial cancers and strongly predicted metastasis and death. *c-MYC* was sufficient to reactivate the ESC-like program in normal and cancer cells [Wong et al., 2008]. The findings suggested that ESCs usually became committed to form adult tissues and once such adult cells became cancerous they turned on the ESC genes that were switched off. Activation of an ESC-like transcriptional program in adult differentiated cells may induce pathological self-renewal characteristic of CSCs [Wong et al., 2008]. It appears that the genes active in both ESCs and CSCs are controlled by a few master regulatory genes, one of which is *c-MYC*.

Ben-Porath et al. [2008] showed that histologically poorly differentiated tumors showed preferential overexpression of genes normally enriched in ESCs. Activation targets of *NANOG*, *OCT4*, *SOX2*, and *c-MYC* are more frequently overexpressed in poorly differentiated tumors than well-differentiated tumors. The ESC signature was also present in glioblastomas and bladder carcinomas. These authors emphasized that they show a previously unknown link between genes associated with ESC identity and the histology of tumors supporting the possibility that these genes contribute to stem cell-like phenotypes shown by many tumors [Ben-Porath et al., 2008].

Several other workers also reported that the cell-signaling pathways such as notch, sonic hedgehog, wnt-catenin, and fibroblast growth factor-2 that regulates normal hESCs are also found to be involved in oncogenesis [Dvorak et al., 2006]. In addition, self-renewal potential, high proliferative capacity, increased telomerase activity, high nuclear to cytoplasmic ratios, and increased expression of antiapoptotic genes are some of the common characteristics that exist between hESCs and cancer cells. Recently, genes specific to embryonic stages were found to be expressed in certain tumor conditions thereby providing a direct molecular link between ESCs and carcinogenesis [Zhang et al., 2006].

EMBRYONIC STEM CELLS AND STATINS

A recent study of statins on mouse embryonic stem cells (mESCs) demonstrated that statins inhibited their cell proliferation and also led to loss of self-renewal capacity. This was mediated by RhoA geranylgeranyl inhibition, and the inhibitory effects were reversible with GGPP substitution [Lee et al., 2007]. In contrast to mESCs, karyotypically normal hESCs were not affected by both hydrophilic and lipophilic statins [Gauthaman et al., 2007] (Fig. 2). However, in the hESC study, cell proliferation was inhibited in a karyotypically abnormal hESC line (that contained duplications of chromosomes 12 and 17) by simvastatin, lovastatin, and mevastatin. A comparison of cancer cell lines with and without 12/17 gene mutations (viz., ovarian adenocarcinoma and colorectal adenocarcinoma, respectively) also demonstrated inhibition of cell proliferation with lyophilic statins (Fig. 2). Additionally, TUNEL-positive cells were observed in the abnormal hESC, ovarian cancer, and colorectal cell lines and the cell cycle assay revealed evidence of apoptosis with simvastatin and lovastatin. Simvastatin was the most potent of the four statins studied. Simvastatin treatment, also up-regulated several "stemness"-related genes located on chromosomes 12 and 17, and some common genes involved in apoptosis (*BAX*, *BCL2*, *P53*) in hESCs, while some of these genes were down-regulated in the abnormal hESC, ovarian cancer, and colorectal cells. Statins therefore are effective against both stem cells and cancer cells and it is possible that statins inhibit cell proliferation in karyotypically abnormal stem and cancer cells via an increase in activity of key apoptotic genes and the suppression of key "stemness" genes on chromosomes 12 and 17 [Gauthaman et al., 2007].

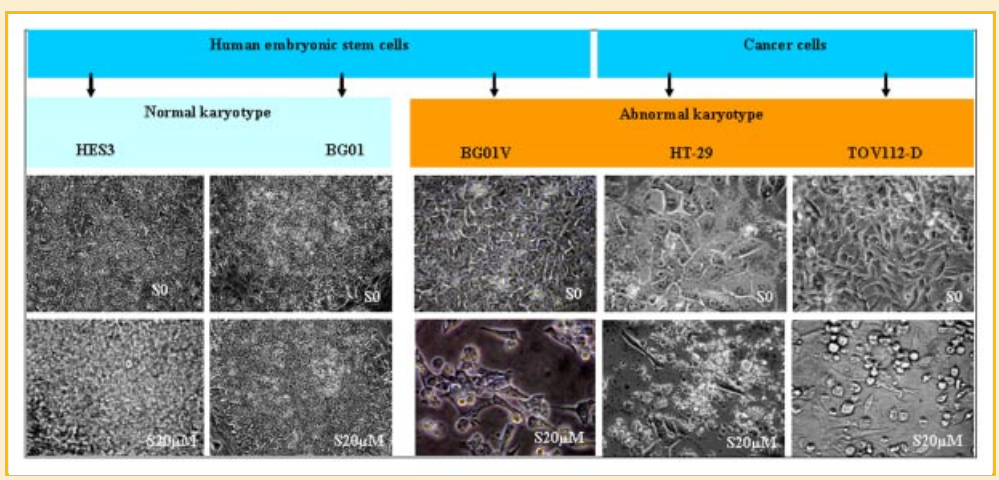

Fig. 2. Effect of simvastatin (20 μM) on karyotypically normal human embryonic stem cells (hESCs) (HES3, BG01) and karyotypically abnormal hESCs (BG01V), human colorectal cancer (HT-29), and human ovarian cancer (TOV-112D) cells following incubation for 72 h. HES3 (ESI, Singapore); BG01, BG01V (BresaGen, USA, Parent and daughter hESC lines); HT-29, TOV-112D (ATCC, USA). S0: Before simvastatin treatment; S20: after 20 μM simvastatin treatment. 40× magnification. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

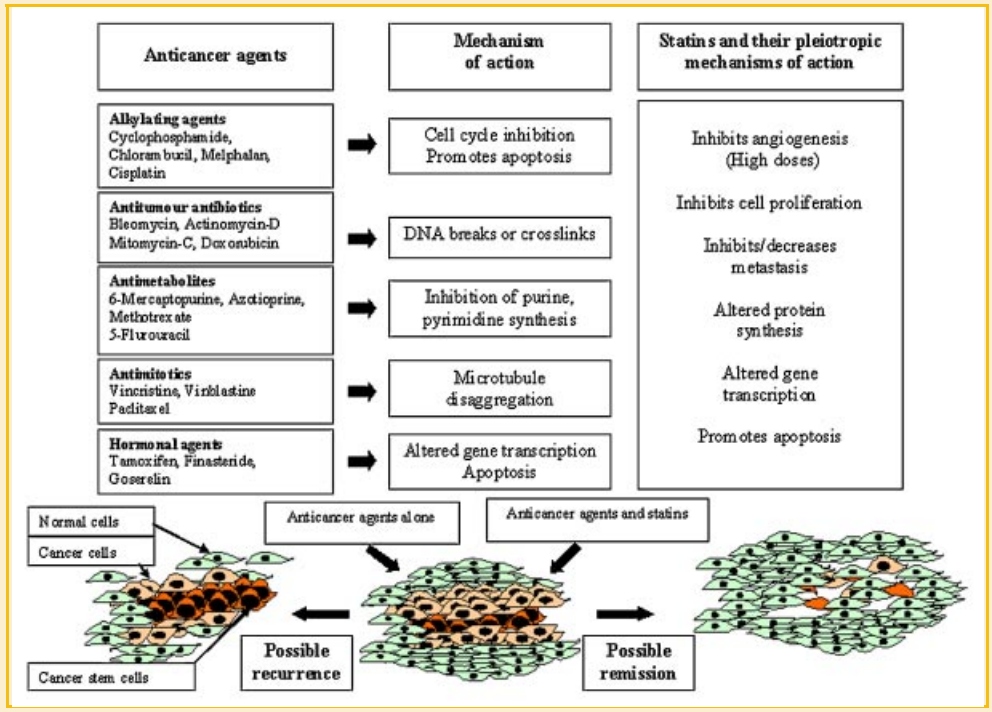

Fig. 3. Elimination of karyotypically abnormal stem and cancer cells. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

These recent findings strengthen the hypothesis that the statins while inhibiting proliferation in hESCs via suppression of "stemness" genes [Gauthaman et al., 2007] may also suppress similar "stemness fingerprints" in CSCs, thus controlling the growth and metastasis of cancer cells.

CONCLUSIONS AND FUTURE DIRECTIONS

It is evident from many studies that statins decrease the incidence of different cancers. As lipid is an essential cellular component, any pharmacological agent that is involved in its modification or regulation is bound to affect various biochemical pathways and subsequent cellular functions. To fully confirm the anticancer effect of statins, the following future studies need consideration.

Apoptosis and inhibition of cancer cell proliferation are found to occur at specific concentrations in vitro. Such doses must be extrapolated to the in vivo clinical situation. A possible clinical scenario that may be explored is to administer the extrapolated dose of statin to the cancer patient at the time of taking a biopsy and observe the course of the tumor in terms of size, function, and histopathology at the time of surgery, to evaluate the effects of the statin in vivo. Statins appear to be cytostatic at lower concentrations and cytotoxic at higher concentrations. Therefore, long-term studies with low concentrations of statins as used for control of hyperlipidemia need to be carried out, rather than acute high concentrations as normally used in vitro, to see if statins will be beneficial as anticancer agents. Combination with other chemotherapeutic agents might extend the anticancer benefits depending on the level of synergism between different compounds (Fig. 3). Studying the effects of statins directly on a CSC population in vitro and on animal models in vivo would help us to clearly identify the mechanism of action of statins on stem cells.

If the anticancer effects of statins were due specifically to cholesterol inhibition, then, future studies using a combination of statins (a cholesterol synthesis inhibitor) and inhibitors of cholesterol absorption from the intestine against different cancers both in vivo and in vitro would bring to light the real benefits of cholesterol inhibitors against cancer. The therapeutic scope of the statins will expand considerably in the coming years particularly due to its emergence as an anticancer agent besides its proven benefits in the management of atherosclerotic cardiovascular diseases.

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